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Comparative transcriptome analysis of high-growth and wild-type strains of *Pyropia yezoensis*

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Abstract – *Pyropia yezoensis* (Ueda) M.S.Hwang et H.G.Choi is a popular edible macro-alga that is found mostly in intertidal zones. It is one of the most economically important seaweed species and has been cultivated extensively in the cold waters of East Asia. Various reports have been published on the isolation and characterization of improved strains of *Pyropia*. However, there are few studies focusing on the molecular basis underlying these mutant strains. In this study, we performed a comparative analysis of whole transcriptomes of wild-type (PyWT) and high-growth (Py500G) strains of *P. yezoensis* using next generation RNA sequencing (RNA-seq). After sequencing, a total of 167,110,896 paired-end reads with a length of 151 nucleotides, were obtained. *De novo* transcriptome assembly and redundancy removal generated 19,441 transcripts. The assembly was annotated in NCBI nr, Swiss-Prot, Pfam, KEGG, GO and KOG databases. To unravel the differences in Py500G and PyWT, we mapped Py500G and PyWT reads to the assembly and calculated the expression levels. In total, there were 454 transcripts that were differentially expressed. Among the differentially expressed transcripts, candidate genes were identified with well-known growth and development functions. This study not only identifies candidate genes responsible for the high-growth phenotype of Py500G, but it also provides more comprehensive genomic data for future research on *P. yezoensis.*

Keywords: *de novo* assembly, differential expression, *Pyropia yezoensis*, red algae, RNA-seq, transcriptome

Introduction

The marine red alga *Pyropia yezoensis* (Ueda) M.S.Hwang et H.G.Choi is found in intertidal zones, where it faces considerable variability in temperature, water content, and light intensity on a daily basis. It is an economically important seaweed because of its pleasant savory taste and widely-recognized nutritional values (Noda 1993, Wenjuan et al. 2010). Numerous mutants have been created, providing valuable resources for scientific research and for the introduction of new cultivars with higher commercial values (Niwa et al. 1993, Xing and Yusho 1997, Su-Juan et al. 2000, Yan et al. 2000, Yan et al. 2004, Li et al. 2008, Niwa et al. 2009, Niwa 2010, Zhang et al. 2011, Park and Hwang 2014, Ding et al. 2016). The novel phenotypes range from pigmentation mutations to high yield, disease resistance, or heat tolerance. However, there are few studies making an in-depth investigation of the molecular basis underlying these mutants.

Previously, weisolated *P. yezoensis* 500G (Py500G) which is a mutated strain generated by gamma irradiation (Lee et al. 2019). The strain exhibited enhanced growth and high-temperature tolerance when compared to the wild type. However, the mutant Py500G was not analyzed at a molecular level. One of the biggest challenges in genetic studies of *P. yezoensis* is the lack of a complete reference genome. It was estimated that its genome is about 260 Mbp in size (Matsuyama-Serisawa et al. 2007). Meanwhile, the current draft genome of *P. yezoensis* is approximately 43 Mbp and composed of 46,634 contigs (Nakamura et al. 2013). To counter this problem, genome-wide transcriptome profiling and *de novo* assembly has been extensively employed to investigate multiple characteristics of this species (Nikaido et al. 2000, Asamizu et al. 2003, Xu et al. 2006, Kitade et al. 2008, Liang et al. 2010, Yang et al. 2011, Sun et al. 2015).

With the aim of studying the molecular characteristics of *P. yezoensis* 500G, we performed transcriptome sequencing using RNA-Seq. RNA-Seq can provide a comprehensive overview of the whole genome expression profile of Py500G. Based on those data, genes that are differentially expressed can be identified to help to determine the mechanism re-

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sponsible for the Py500G phenotype. The study will not only help to unravel the molecular mechanism of the high growth rate of Py500G but will also provide more comprehensive genomic data for a better understanding of the metabolic processes of *P. yezoensis*.

Materials and methods

Materials and cultivation condition

Whole gametophytes of Py500G, which was developed in our laboratory, and the original wild type strain PyWT from the Seaweed Research Center (National Fisheries Research and Development Institute, South Korea) were used in the study. Gametophytes were cultivated in modified Grund medium (MGM) (McLachlan 1973) under 80 μmol photons m^{-2} s^{-1} and a photoperiod of 10 L:14 D at 10 °C in a growth chamber. Algal cultures were continuously aerated with filter-sterilized air and the medium was changed on a weekly basis.

RNA extraction and sequencing

Total RNA was isolated from the gametophytes of Py500G and PyWT algae using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. The quality of the processed RNA samples was confirmed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and by agarose gel electrophoresis. Total RNA was treated with DNase I and poly-dT oligonucleotide-coated magnetic beads to elute poly-(A)+ mRNA. Purified mRNA was fragmented using a DNA fragmentation kit (Ambion, Austin, TX, USA) prior to cDNA synthesis. The cleaved mRNA fragments were primed using random-hexamer primers and reverse transcriptase (Invitrogen) was used to synthesize first-strand cDNA. Second-strand cDNA synthesis was performed using RNase H (Invitrogen) and DNA polymerase I (New England Biolabs, Ipswich, MA, USA). Subsequently, end-repair of double-stranded cDNA was performed using T4 DNA polymerase, the Klenow fragment, and T4 polynucleotide kinase (New England Biolabs). The end-repaired cDNA was ligated to the Illumina paired-end (PE) adapter oligonucleotide-mix using T4 DNA ligase (New England Biolabs) at room temperature for 15 min. Suitable fragments were then sequenced in a PE pattern on an Illumina HiSeq 2000 instrument (Illumina, Inc., San Diego, CA, USA). Sequencing data were transformed by base calling into raw reads and stored in fastq format.

Data pre-processing, *de novo* assembly and redundancy removal

The raw reads were deposited in NCBI Sequence Read Archive under accessions from SRR5891396 to SRR5891400. Read quality was assessed using FastQC 0.11.5 (Andrews 2010). Raw data were first pre-processed to remove adapter sequences and low-quality data using Trimmomatic 0.36 (Bolger et al. 2014). The processed, clean paired-end reads were then used for *de novo* assembly using the short read assembling program Trinity v2.4.0 (Grabherr et al. 2011) with the default k-mer of 25. The Trinity assembler combined reads to form longer overlapping contigs without gaps. We then used TransDecoder (Haas and Papanicolaou 2016) to identify candidate coding regions within transcript sequences and to eliminate transcripts which open reading frames (ORFs) encoded less than 100 amino acids. Finally, CD-HIT (Li and Godzik 2006, Fu et al. 2012) was used to remove redundancy from the assembly. Specifically, the ORF sequences generated by TransDecoder were clustered into groups of 90% identity using CD-HIT. Transcripts containing the longest ORFs of each cluster were kept.

Functional annotation

To understand the functions of the transcripts in the assembly, we annotated transcripts by following the Trinotate workflow (Bryant et al. 2017) with some modifications. The candidate ORF sequences were used as queries for a homology search against NCBI nr and Swiss-Prot databases (BLASTP, e-value cut-off of 1E-5). BLAST+ program (Camacho et al. 2009) was used to search for homologous sequences in Swiss-Prot and DIAMOND aligner (Buchfink et al. 2015) was used to search the NCBI nr database. Protein domains of transcripts were retrieved from the Pfam database using hmmscan (Finn et al. 2011) (e-value cut-off of 1E-5). Pathway annotation at the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto 2000) was performed using the KEGG Automatic Annotation Server (KAAS), with the Single-directional Best Hit method and the gene data set for eukaryotes. Finally, the stand-alone program, InterProScan (Zdobnov and Apweiler 2001), was used to retrieve gene ontology (GO) terms by scanning query sequences for matches against the InterPro protein signature databases.

Differential expression analysis

Bowtie2 (Langmead and Salzberg 2012) was used to map clean reads from Py500G and PyWT to the assembly. Abundance estimation and differentially expressed gene analysis were performed using RSEM (Li and Dewey 2011) and EdgeR (Robinson et al. 2010), respectively. Transcripts with more than a 2-fold difference in expression between Py500G and PyWT and with a false discovery rate (FDR) of less than 0.05 were considered differentially expressed.

RT-qPCR validation

Total RNA was extracted from Py500G and PyWT gametophyte thalli after 4 weeks of culturing. The algae were powdered in liquid nitrogen and RNA was extracted using a RNeasy Mini Kit (Qiagen, Hilden, Germany) and DNase I treatment. cDNA was synthesized using a Transcriptor First Strand cDNA Synthesis Kit (Roche, Basel, Switzerland) with oligo-dT primers.

qRT-PCR was performed using a SYBR Premix Ex Taq™ II Kit (TaKaRa, Kusatsu, Japan) in a 20 μL reaction volume,

containing 10 μL of 2×SYBR Green Mastermix (TaKaRa), 2 μL of forward and reverse primers, 1 μL of cDNA, and 7 μL of ddH2O. Reactions were performed using the following program: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, 55 °C for 20 s, 72 °C for 20 s, and with reading fluorescence signal detection, and then 1 cycle of 95 °C for 15 s, 60 °C for 60 s, and 95 °C for 15 s. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control RNA (Wu et al. 2013). The qPCR primers used in this study are included in On-line Suppl. Tab. 1.

Results

De novo sequencing and assembly of *P. yezoensis* transcriptome

The transcriptome of two *P. yezoensis* strains, Py500G and PyWT, were sequenced using an Illumina HiSeq 2000 Sequencing instrument. RNA sequencing of gametophyte thalli generated 56,712,121 and 110,398,775 paired-end reads for Py500G and PyWT, respectively. After preprocessing to remove low-quality reads and adapter sequences, 87.84% of the raw data remained for *de novo* assembly and subsequent analysis (Tab. 1).

De novo transcriptome assembly was performed using Trinity. Since this new assembly contained a large quantity of redundant sequences, which can hinder downstream comparative analysis (Ono et al. 2015), we clustered peptides translated from the Trinity transcript ORFs into groups of 90% and kept only those transcripts encoding the longest peptides in each group. As a result, we obtained 19,441 non-redundant transcripts in the assembly with an average GC content of 67.54% (Tab. 2).

Tab. 1. The transcriptome sequencing of *Pyropia yezoensis*. C1, C2, and C3 are replicates of PyWT and M1 and M2 are replicates of mutant Py500G.

	Sample ID	Raw reads	Clean reads	GC%
PyWT	C1	37,089,092	31,978,088	63
	C2	34,298,948	29,701,219	63
	C3	39,010,735	33,847,405	63
Py500G	M1	37,809,087	34,155,831	63
	M2	18,903,034	16,885,815	63

Tab. 2. Data for *de novo* transcriptome of *Pyropia yezoensis*. GC percentage (GC%), the minimum contig length to cover 50 percent of the genome (N50), minimum, maximum, median and average length of the assembled transcripts, and the total base count of the assembly.

Statistic	Value
Number of transcripts	19,441
GC%	67,54
N50	1,898
Minimum length	297
Maximum length	13,853
Median length	888
Average length	1,295
Total assembled bases	25,183,681

Functional annotation

Functional annotation facilitates downstream analyses and accelerates the discovery of new genes. The numbers of transcripts annotated in each database are listed in Table 3. We annotated transcripts based on NCBI nr, Swiss-Prot, Pfam, KEGG, GO, and KOG databases. More than 77.5% of total transcripts were annotated in at least one database. 98.8% of annotated transcripts had homologous sequences in NCBI nr, of which 68% were from *Porphyra umbilicalis* Kützing (Fig. 1A). In contrast, only 46.7% of annotated transcripts had homologous sequences in the manually curated Swiss-Prot database. KEGG pathway annotation provides further understanding of the biological functions of transcripts (Fig. 1B). This analysis revealed 2190 KEGG orthology (KO) identifiers associated with 3,104 transcripts in the assembly. Finally, based on their GO annotations, transcripts were classified into Cellular Component, Molecular Functions, or Biological Process groups (Fig. 1C).

Differential expression analysis

We mapped clean reads to the assembly and then estimated gene abundance for each sample. Using the log2-transformed CPM (counts per million) data, we examined the correlation between pairs of samples by calculating Pearson's correlation coefficients. All samples were highly correlated with each other as the Pearson correlation coefficients (r) ranged from 0.97 to 0.99 across all pair-wise comparisons (On-line Suppl. Fig. 1).

With an FDR cut-off of 0.05, a total of 454 transcripts were identified as differentially expressed transcripts between Py500G and PyWT. These consisted of 192 up-regulated and 262 down-regulated transcripts in Py500G. Out of these differentially expressed transcripts, only 199 were annotated in at least one database and not recorded as "hypothetical protein" or "unknown protein". The list of these annotated transcripts is included in On-line Suppl. Tab. 2.

Out of 199 annotated DEGs, we identified a list of genes from groups of functions well-known for their association with growth and development (Tab. 4). These included genes involved in photosynthesis, nitrogen uptake and assimilation, and cellular homeostasis. Furthermore, based on KEGG pathway annotations and manual curation, we also demonstrated the interaction among these candidate transcripts in Fig. 2.

Tab. 3. Functional annotations of all *Pyropia yezoensis* transcripts according to six public databases.

Database	Number of transcripts	Percentage (%)
Annotated in NCBI nr	14,894	76.6
Annotated in Swiss-Prot	7,039	36.2
Annotated in Pfam	7,368	37.9
Annotated in KEGG	3,104	16.0
Annotated in GO	6,151	31.6
Annotated in KOG	7,154	36.8
Annotated in at least one database	15,075	77.5

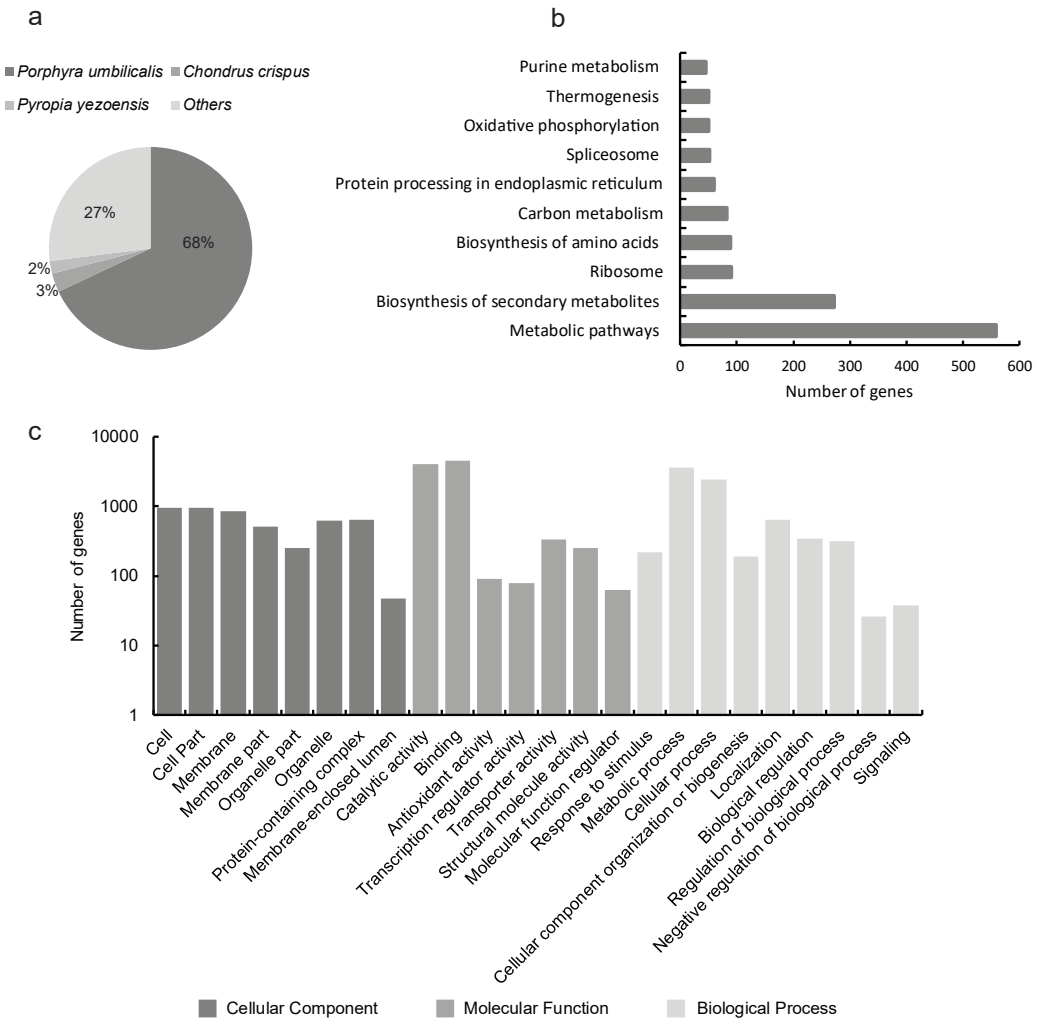

Fig. 1. Functional annotation of *Pyropia yezoensis* 500G assembled transcripts: a – the species distribution of the NCBI nr annotation which is shown in percentage, b –KEGG pathway classification, c – gene ontology classification which is summarized in three categories: cellular component, molecular function, and biological process.

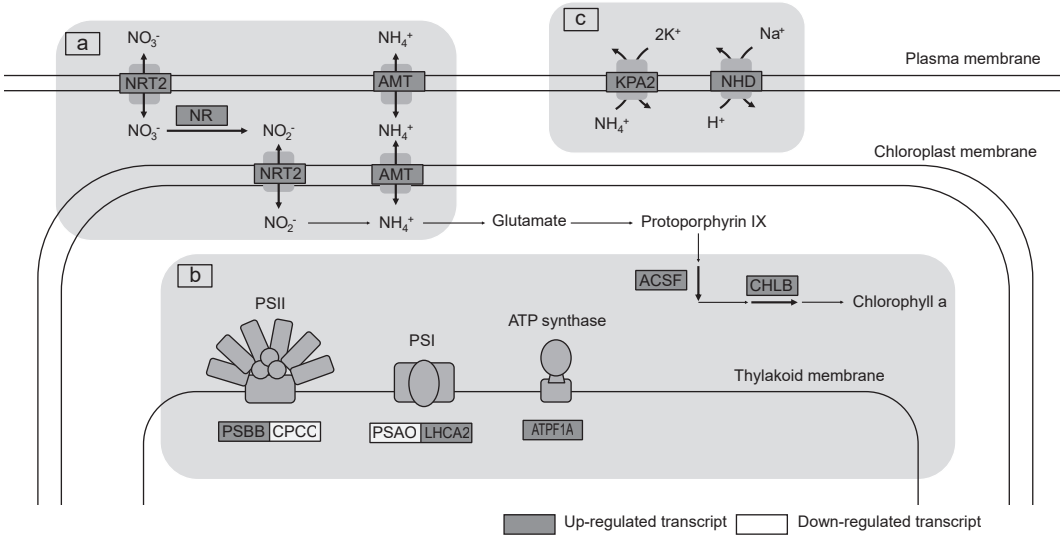

Fig. 2. Schematic representation of altered pathways relevant to the proposed candidate genes: a – nitrogen uptake and assimilation, b – photosynthesis, c – cellular homeostasis. Up-regulated transcripts are in dark-grey and down-regulated are in light-grey cells. ACSF – magnesium-protoporphyrin IX monomethyl ester (oxidative) cyclase, AMT – ammonium transporter, ATPF1A – F-type H+-transporting ATPase subunit alpha, CHLB – light-independent protochlorophyllide reductase subunit B, CPCC – phycobilisome linker polypeptide, KPA2 – sodium/potassium-transporting ATPase subunit alpha, LHCA2 – light-harvesting complex I chlorophyll a/b binding protein 2, NR – nitrate reductase; NRT – nitrate/nitrite transporter, PSAO – photosystem I subunit O, PSBB – photosystem II CP47 chlorophyll apoprotein.

Tab. 4. List of differentially expressed *Pyropia yezoensis* genes involved in photosynthesis; nitrogen uptake and assimilation; and cellular homeostasis. These genes are potential candidates for further research into the molecular basis of mutant *Pyropia yezoensis* Py500G. Expression difference of each transcript between Py500G and wild type PWT is indicated as log-2-fold change (logFC) along with its correspondent false discovery rate value (FDR).

Function groups	Transcript ID	Description	logFC	FDR
Photosynthesis	DN6375_c0_g1_i10	psbB; photosystem II CP47 chlorophyll apoprotein	1.136642	0.004743
	DN6375_c0_g9_i1	ATPF1A, atpA; F-type H+-transporting ATPase subunit alpha [EC:3.6.3.14]	1.956919	7.27E-07
	DN3901_c0_g1_i1	E1.14.13.81, acsF, chlE; magnesium-protoporphyrin IX monomethyl ester (oxidative) cyclase [EC:1.14.13.81]	1.731197	0.001166
		chlB; light-independent protochlorophyllide reductase subunit B [EC:1.3.7.7]	1.731197	0.001166
	DN6028_c1_g1_i1	Phycobilisome 31.8 kDa linker polypeptide, phycoerythrin-associated, rod	–1.92291	0.006047
Nitrogen uptake and assimilation	DN5456_c1_g2_i2	amt, AMT, MEP; ammonium transporter, Amt family	1.290219	0.017711
	DN6680_c6_g4_i1	NRT, narK, nrtP, nasA; MFS transporter, NNP family, nitrate/nitrite transporter	1.186616	0.003007
	DN5863_c0_g1_i1	High-affinity nitrate transporter 2.2	1.776852	2.04E-05
	DN6102_c1_g1_i1	NRT, narK, nrtP, nasA; MFS transporter, NNP family, nitrate/nitrite transporter	1.483347	1.08E-05
	DN7095_c1_g5_i2	High affinity nitrate transporter 2.6	1.438567	1.31E-05
	DN7094_c5_g3_i1	Nitrate reductase [NADH]	1.185227	0.038693
Cellular homeostasis	DN6953_c7_g1_i2	ppa; inorganic pyrophosphatase [EC:3.6.1.1]	1.548605	1.99E-08
	DN4792_c0_g1_i1	Sodium/proton antiporter 1	1.636608	1.86E-05
	DN5850_c2_g4_i2	PyKPA2; ATP1A; sodium/potassium-transporting ATPase subunit alpha [EC:3.6.3.9]	1.101731	0.036709

RT-qPCR validation

To confirm the expression of transcripts identified by RNA-seq data, we randomly chose 5 differentially expressed transcripts for RT-qPCR validation. One of these transcripts contained 2 different ORFs, which were *ACSF* and *CHLB*. Of these 5 transcripts, 3 showed the same pattern of expression as in RNA-seq data (Fig. 3). The transcript containing 2 ORFs also showed consistent results between RT-qPCR and RNA-seq analyses. However, the 2 ORFs showed different levels of expression, despite belonging to the same transcript. This may be the result of different qPCR primer efficiencies.

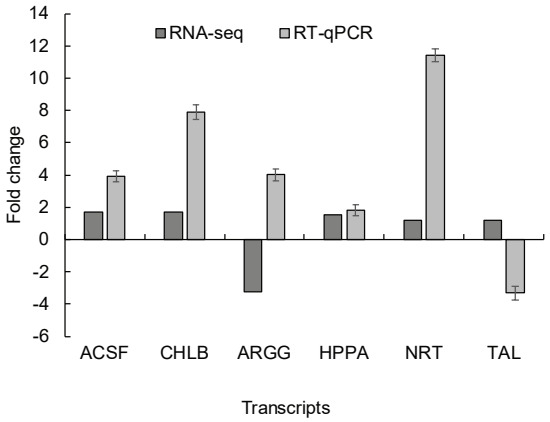

Fig. 3. Validation of RNA-seq data with RT-qPCR. ACSF – magnesium-protoporphyrin IX monomethyl ester (oxidative) cyclase, CHLB – light-independent protochlorophyllide reductase subunit B, ARGG – argininosuccinate synthase, HPPA – K(+)-stimulated pyrophosphate-energized sodium pump, NRT – nitrate/nitrite transporter, TAL – transaldolase.

Discussion

The aim of this study was to identify candidate genes for further study of the mechanisms underlying the high-growth phenotype of *P. yezoensis* 500G. We approached this by investigating the differences in gene expression between Py500G and PyWT under normal culture conditions, using RNA-seq. In total, 454 transcripts were differentially expressed between Py500G and PyWT. These transcripts belonged to various functional groups and were all potentially involved in determining the high growth rate of Py500G. Further studies focusing on these candidates, especially on the previously uncharacterized genes, may reveal novel molecular mechanisms of the valuable feature in red algae.

Three plastid transcripts involved in photosynthesis were up-regulated in Py500G. The first transcript was *PSBB*, which encodes one of the photosystem II components. The second transcript was *ATPF1A*, which encodes the CF1 alpha subunit of chloroplast ATP synthase. The final transcript contained 2 ORFs, *ACSF* and *CHLB*, both of which are involved in chlorophyll biosynthesis. The protein encoded by *ACSF* catalyzes the formation of the isocyclic ring of chlorophyll. Meanwhile, *CHLB* encodes one of the subunits

of light-independent protochlorophyllide oxido reductase (DPOR), which allows the efficient synthesis of chlorophyll in the dark or under low light conditions (Shui et al. 2009). *ACSF* and *CHLB* are not only found in *P. yezoensis*, but are also located adjacent to each other in the plastid genomes of other *Pyropia* species (Wang et al. 2013, Hughey et al. 2014). Unlike the situation with LPOR, which is a light-dependent POR, few studies have been published on the regulation of DPOR in algae. However, the high levels of expression of the transcript containing *ACSF* and *CHLB* were expected to promote chlorophyll synthesis, and along with the up-regulation of *PSBB* and *ATPF1A*, subsequently increase photosynthesis in Py500G.

One transcript involved in photosynthesis was found to be down-regulated in Py500G. It was annotated as a phycobilisome linker protein. In contrast with the 2 photosynthesis transcripts mentioned above, which shared 100% sequence identity with previously reported *P. yezoensis* genes, this transcript showed low homology. In fact, the most homologous sequence in *P. yezoensis* was a plastid phycobilisome linker protein (accession: YP_536944.1) with identity as low as 28%. However, there was a transcript in the mapping reference that shared 100% identity with YP_536944.1, but this transcript was not differentially regulated.

Dissolved inorganic nitrogen in seawater includes nitrate, nitrite, and ammonium and is a key factor for the growth, development, and quality of *P. yezoensis* thalli (Kakinuma et al. 2017). Two proteins that play crucial roles in nitrogen uptake, ammonium transporter (AMT) and nitrate transporter (NRT) were transcriptionally up-regulated in Py500G. The *amt* transcript was identical with the reported *PyAMT1* in *P. yezoensis*, which is highly regulated in response to external/internal N-status (Kakinuma et al. 2017). There were up to 4 nitrate transporter transcripts that shared 94 to 99% sequence identity with the *PyNRT2* gene, which has also been previously reported in *P. yezoensis* (Kakinuma et al. 2008). These 4 transcripts were not full-length, were not designated as isoforms of each other by Trinity, and 3 of them overlapped. Additionally, in the same study, the authors used Southern blotting analysis to show that there is only one NRT2-encoding gene in the *P. yezoensis* genome. Hence, these 4 transcripts may be transcribed from one gene encoding for PyNRT2, which is responsible for high-affinity nitrate transport across the plasma membrane. Interestingly, besides the two nitrogen transporters, we also identified another up-regulated transcript that encodes a key enzyme in nitrogen assimilation. Nitrate reductase catalyzes the reduction of nitrate to nitrite, consuming NADPH as the reducing equivalent, in the rate-limiting step of nitrate assimilation in plants and algae (Sakihama et al. 2002). All these results indicated that Py500G had a higher rate of nitrogen uptake and assimilation than PyWT. Besides, there is no report of nitrate reductase in *P. yezoensis*, except for a study on enzyme isolation and purification (Nakamura and Ikawa 1993). The nucleotide sequence of nitrate reductase identified in this study will be valuable for further studies on nitrate uptake and assimilation in red algae.

To grow and develop, especially in a high-salt environment like seawater, it is critical for *P. yezoensis* to establish and maintain ion and pH homeostasis in all cellular compartments. To accomplish this, cells need to activate various types of membrane transporters, of which proton, sodium, and potassium transporters are the most important. In Py500G, 3 transcripts encoding a sodium pump (KPA), a sodium/proton antiporter (NHD), and a proton-pumping inorganic pyrophosphatase (VPPA) were found to be up-regulated. These 3 transcripts shared 99 – 100% identity with previously identified genes or gene fragments of *P. yezoensis*, i.e. *PyKPA2* (Uji et al. 2012a), *PyNhaD* (Uji et al. 2012b), and a 639-bp mRNA from the *VPPA* gene (Pérez-Castiñeira et al. 2002). Both PyKPA2 and PyNhaD have previously been shown to be expressed in gametophyte thalli (Uji et al. 2012a, b), which is consistent with our study. As for the *VPPA* gene, our transcriptome data showed that it was a putative full-length transcript, with an ORF expected to encode a 1079-aa protein.

Other functional groups were also identified from the list of 199 DEGs (Tab. 5). However, transcripts belonging to these functions did not show a consistent trend of expression or were poorly characterized in the literature. It was challenging to assign to these groups their exact biological significance.

Transposable elements (TEs): Various TEs were found to be up-regulated in Py500G. Homology search showed 2 of the total 10 transcripts exhibited 37% identity to a previously characterized retrotransposon in *P. yezoensis* (Peddigari et al. 2008). It is unclear what the significance of the high expression of TEs in Py500G is. TEs make a great contribution to the genomes of red algae. It was estimated that 43.5% of genome of *Porphyra umbilicalis* consists of TEs (Lee et al. 2018). This number was even higher in *Chondrus crispus* where TEs accounted for up to two third of its genome (Collén et al. 2013). The presence of various TEs in the DEGs list might partly be because of the high proportion of TEs in the genome.

ABC transporters: ABC transporter families are one of the largest groups of transporter in plants and involved in various cellular processes, e.g. detoxification, pathogen response, surface lipid deposition, etc. (Kang et al. 2011). It was also shown that many of the *Arabidopsis* ABC transporter sequences were present in red-alga genomes (Schulz and Kolukisaoglu 2006). In Py500G, 3 sequences of ABC transporters were differentially expressed: one up-regulated transcript *AB7G* and 2 down-regulated *AB16G* and *AB27G*. However, little is known about the functional characteristics of these 3 transporters in marine algae.

Protein kinases: Kinases constitute a very large group of protein families and are crucial components of different biological pathways. 7 transcripts encoding for protein kinases were differentially expressed. Most of these transcripts were not found in *Pyropia* before, except for a type II topoisomerase that showed from 90 to 100% identity with five *Pyropia* algae species: *P. tenera*, *P. onoi*, *P. suborbiculata*, *P. haitanensis* and *P. dentata* (Shimomura et al. 2000).

Tab. 5. Additional differentially expressed genes of mutant *Pyropia yezoensis* Py500G. These genes did not show consistent trends of expression, hence imposed challenges in interpreting their biological relevance. Expression difference of each transcript between Py500G and wild type PWT is indicated as log-2-fold change (logFC) along with its correspondent false discovery rate value (FDR).

Function groups	Transcript ID	Description	logFC	FDR
Transposable elements	DN10049_c0_g1_i1	Transposable element Tc1 transposase	4.097839	0.000261
	DN4967_c0_g2_i1	Tigger transposable element-derived protein 4	6.277311	1.25E-15
	DN5421_c0_g1_i1	Putative transposon Ty5-1 protein YCL074W	4.348569	2.79E-05
	DN5535_c1_g1_i1	Tigger transposable element-derived protein 2	3.165459	0.004952
	DN5829_c0_g1_i2	Transposable element Tcb2 transposase	10.14786	2.23E-13
	DN6460_c2_g1_i2	Transposable element Tcb1 transposase	3.315518	0.014183
	DN6460_c2_g1_i2	paired box protein and transposase domain containing protein [Lasius niger]	3.315518	0.014183
	DN6471_c4_g2_i4	Retrovirus-related Pol polyprotein from transposon TNT 1-94	5.732638	1.86E-06
	DN6471_c4_g2_i8	Retrovirus-related Pol polyprotein from transposon TNT 1-94	13.1111	1.04E-49
	DN6704_c0_g1_i2	Retrotransposable element SLACS 132 kDa protein	3.31226	0.015422
ABC transporters	DN5923_c14_g2_i1	ABC transporter G family member 7	2.755925	0.007972
	DN7183_c2_g1_i1	ABC transporter G family member 16	–7.63038	0.040887
	DN7593_c6_g1_i4	ABC transporter G family member 27	–8.14906	0.022452
Protein kinases	DN3899_c0_g1_i2	protein kinase, PfEST homolog, putative [Eimeria brunetti]	8.799801	1.55E-07
	DN4061_c0_g1_i1	Serine/threonine-protein kinase	–2.0927	0.029542
	DN5245_c0_g1_i1	ppnK, NADK; NAD+ kinase [EC:2.7.1.23]	1.681926	0.000133
	DN6168_c0_g3_i1	SNF1-related protein kinase regulatory subunit beta-2	1.014229	0.018158
	DN6277_c0_g1_i2	TOP2; DNA topoisomerase II [EC:5.99.1.3]	–2.70388	2.83E-07
	DN6277_c8_g1_i1	Serine/threonine-protein kinase	1.36869	0.007944
	DN6652_c0_g2_i1	E2.7.1.20, ADK; adenosine kinase [EC:2.7.1.20]	–2.98125	0.000642

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